LABORATORY ASPECTS OF *TRICHOMONAS* INFECTIONS*†

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In discussing the laboratory aspects of *Trichomonas* infections, I hope to show that it is often possible to obtain valuable information about one strain by inference from the behaviour of others, and that studies of other members of the group may, in the end, increase our knowledge of *T. vaginalis*.

The majority of workers studying the trichomonads have concentrated their attention on the species known to be pathogenic: *T. vaginalis*, *T. foetus*, and *T. columbae* (or *T. gallinae*). A fourth species which deserves more attention is *T. hominis*, which is found in the bowel, and is sometimes present in large numbers in the stools of patients with mild diarrhoea, particularly in the tropics. There is no evidence that it is pathogenic in the bowel, nor indeed, although sometimes held to be responsible, is there any evidence that it can cause vaginitis. There are, however, some curious contradictions in the literature on this species to which I should like to draw your attention later.

In the last few years I have maintained cultures of the four strains mentioned above, and have gained some familiarity with their behaviour, similarities, and differences.

To start with *T. vaginalis* itself, it is about 10 years since the cultivation of this organism without bacteria became a relatively simple matter, thanks to the antibiotics and to improved media. In that time a great deal has been achieved. Media have been improved still further, and probably the best medium at present available is that described by Feinberg (1953). These improved media not only provide for the rapid growth of the protozoa and the development of a very large final population, but will also permit the survival and growth of very small inocula. Thus I have found that even as few as an estimated 5 to 10 organisms, when inoculated into tubes containing 10 ml. medium, will grow and give a macroscopically detectable growth, provided the culture is incubated for a sufficient length of time.

This brings us to a point where laboratory experience is in conflict with the clinical experience recorded by Lanceley (1954). I do not know what is the smallest number of protozoa that must be present in a specimen before we are able to record a positive result by microscopic examination, but I suspect it would have to be greater than 10 per ml. Theoretically cultivation should be a more delicate test than microscopy, yet Dr. Lanceley does not find this to be so. I think the most likely explanation of this is that the wild strains of *T. vaginalis* which the clinician encounters do not survive or grow so well as the adapted strains which we use in experimental work.

There remain two avoidable sources of error in cultivation experiments. If the inoculum is very small, say less than one hundred organisms, the culture may require as long as 5 to 6 days' incubation before growth is readily detectable. It would be a safe general rule never to discard any culture as negative until it had been incubated for a week. Secondly, media for the bacteria-free cultivation of trichomonads rely for their success on the maintenance of a low oxidation reduction potential by means of chemicals such as cysteine hydrochloride, sodium thioglycollate, etc. Media of this type do not keep well and when stale do not support growth. It follows, therefore, that if medium which is near the end of its useful life is inoculated and kept for a further week it may fail to give a positive result although there were in fact a few living protozoa in the original inoculum.

It might be found useful in clinical practice to employ two types of medium in parallel. The first, one of the highly specialized media, such as that described by Feinberg (1953) with antibiotics added, the second a simple horse-serum Ringer medium, in which the associated bacteria would be allowed to grow. The results of such an investigation might be most useful and enlightening.

I carried out some experiments to determine the effect of the size of inoculum on the rate of growth

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of trichomonads, using the three strains *T. foetus*, *T. columbae*, and *T. vaginalis* in a modified Feinberg medium. It was interesting to note that the general pattern of growth was the same for each strain. The average generation time over the whole period of growth was about 6 to 8 hours, a striking difference when compared with bacteria which, during the logarithmic phase of growth, have a generation time of about 20 minutes. No lag phase was shown by any of the strains tested, and the generation time did not appear to be affected by the size of the inoculum.

Dr. Lanceley has mentioned the role of *T. foetus* in bovine infertility and suggested to you that we know little about the potentiality of *T. vaginalis* in relation to human fertility. I should like to mention something which is, perhaps, less well known, that is the existence of two serologically distinct strains of *T. foetus*. These are known as the Belfast and Manley strains. You might wonder, in view of this, if there are any serological varieties of *T. vaginalis*. I do not know of any having been described, but there is no reason to suppose that we should not be able to discover serologically distinct strains if we investigated the problem carefully enough.

T. columbae.—I first isolated the strain I have from a pigeon which had been brought into the Liverpool School of Tropical Medicine in a dying condition. Briefly, the protozoa parasitize the crops of young birds, so producing an appreciable mortality. The survivors become chronically infected without obvious ill-effect. Such birds may subsequently infect their own nestlings in the process of crop feeding. The disease is of some importance to pigeon fanciers and there is no very effective treatment.

Mr. R. B. Griffiths, the parasitologist who sent me the material from which this strain was cultured, suggested that I should use the cultures to produce experimental infections, and then try the effect of treatment with 2-amino-5-nitrothiazole, a drug which was successfully employed in the treatment of turkeys infected with another flagellate, *Hexamita meleagridis*. This was done, in a very small series. The treatment, which consisted of the oral administration of the drug, appeared to be successful, but the work was started too late in the year to ensure a supply of young birds and I was forced to abandon it. During the summer vacation the birds remained well though untreated, and when next examined they were found to be harbouring the protozoa, though now completely symptom-free. It appeared from these findings that the infection had only been suppressed by this drug in the doses employed.

In vitro experiments showed several interesting features. It was clear that even in effective concentration the drug had no immediate lethal action on the protozoa for under the microscope the flagellates continued to show active motility for more than an hour although suspended in an effective concentration.

It is interesting to note in contrast that Macdonald and Tatum (1948a) used the ability of the material under test to immobilize an active culture in 15 minutes as a screening test for drugs likely to be useful in treatment.

In another series of experiments it appeared that no lethal effect was observed in less than 4 hours' exposure to a lethal concentration. The minimal inhibitory concentration as determined *in vitro* was dependent upon the size of the inoculum and varied from 100 to 25 μg./ml. However, 100 μg./ml. was effective in all tests carried out. It seemed that the behaviour of this drug was very much more like that of a metabolic poison such as the antibiotics or sulphonamides than most therapeutic agents used in the treatment of trichomoniasis. *T. columbae*, *T. vaginalis*, and *T. foetus* all showed the same sensitivity to this drug *in vitro*.

Further investigations were postponed until I could again obtain young pigeons of the appropriate age, and before the work was resumed Stabler and Mellentin (1953) published a paper on the treatment of experimentally infected pigeons, using antibiotic mixtures, sulphonamides, and 2-amino-5-nitrothiazole (A.N.T.). The latter proved to be very successful and was, in fact, life-saving. No relapses were recorded. The other treatments were quite useless. I have no doubt that in America the importance of these observations will have been quickly realized and that all the ingenuity of the professional chemist is now being exerted to modify this drug in order to obtain some more rational mode of treatment for trichomoniasis.

Unfortunately, 2-amino-5-nitrothiazole is somewhat toxic and probably could not be used in man in its present form—at least not without very careful trial. However, it is, I feel, very encouraging to see the emergence of such a promising therapeutic agent after the long procession of protein poisons and noxious chemicals which have been used to treat trichomoniasis. A successful agent will be welcomed not only by the appropriate sections of the medical world but also by the veterinary profession, for there is still no way of treating an infected bull which will eradicate *Trichomonas foetus* from the prepuce.

T. hominis.—All the strains which have so far been discussed can be grown in pure culture, and such bacteria-free cultures have many advantages in subsequent research. It is, therefore, all the more curious that the problem of obtaining similar bacteria-free cultures of *T. hominis* has not yet been solved.

I set out some time ago to make a serological comparison of a number of important trichomonads, and *T. hominis* proved to be a stumbling block in that it would not grow in the medium which supported a profuse growth of the other strains. This strain was first isolated from faeces in a simple two-phase medium consisting of an inspissated serum slope overlaid with a 1:8 dilution of horse serum in Ringer solution. In this medium it has subsequently been maintained in the presence of bacterial associates. Reference to the literature was not very helpful. Stabler, Feo, and Rakoff (1941) had used a culture containing bacteria for their inoculation experiments and hence they must have failed to get a pure growth of the protozoa. Macdonald and Tatum (1948b), on the other hand, claimed to have grown *T. hominis* free from bacteria in a simple medium, but this would not grow my strain of this organism. Adler (1951) described a medium which made use of phage-lysed *B. coli* in a sterile medium, but in this I was unable to obtain continuous serial growth. However, in a personal communication, Adler stated that he had found wide differences in the growth requirements of various strains of *T. hominis*; some grew well in simple media without bacteria, others were difficult to grow. It appeared that I had got hold of a difficult strain. I have recently had some success in the growth of this particular strain in a sterile medium, but only if killed bacteria are provided. These results are very conflicting, but there is an even more astonishing observation in the paper of Macdonald and Tatum (1948b) quoted above. These authors set out to compare serologically *T. vaginalis*, *T. hominis*, and *T. foetus*, both directly and by cross-absorption tests. Their results show a complete serological identity between *T. vaginalis* and *T. hominis*—which to me is most surprising. Here indeed is a strange mix-up. Adler observed variation in the growth requirements of various strains of *T. hominis*, some growing easily on simple media, others being much more exacting. More interesting still, Adler commented in a personal communication: "*T. hominis* is readily distinguished serologically from *T. vaginalis*", an observation which one would expect and which seems quite reasonable. It is difficult to think of any explanation which will fit all the facts. The two strains can be distinguished on morphological grounds. Wenrich (1944) gives a clear account of the characteristic feature of each as seen in stained preparations. But, despite the several definite distinguishing features, it is by no means easy to identify trichomonads. Careful study under the dark-ground microscope is probably the best method, so long as the difficulty of too active movement of the flagellates can be overcome without causing distortion. Staining methods are ideal but difficult. Incorrect fixation may result in the flagella being either broken off or wrapped around the body of the organism.

Clearly a great deal more information is needed before the problem can be solved. There are so many differences between *T. vaginalis* and *T. hominis* that it is very difficult to accept their serological identity without further evidence.

If varieties of *T. hominis* exhibiting such wide biological differences as various authors claim do exist, it may be asked whether it is possible that some of them can infect the vagina. Certainly all the evidence at present available is against this possibility. Perhaps when we solve the problem of the cultivation of *T. hominis* we shall be able to add to the evidence that trichomonas vaginitis never originates from the bowel. I believe any information we can obtain about trichomonads is valuable and will help towards the ultimate solution of our particular medical problems.

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